Constituents of *Gaillardia* Species. V. Isolation and Structure of Spathulin[1,2]

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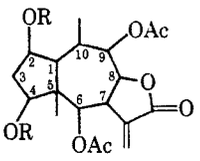
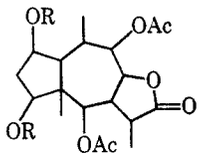
The structure of spathulin, a new pseudoguaianolide from *Gaillardia spathulata* Gray, has been elucidated. Spathulin has also been isolated from *Gaillardia arisatta* Pursh., *Gaillardia mexicana* Gray, and the hybrid *Gaillardia grandiflora*. Flexuosin A has been isolated from *Gaillardia parryi* Greene. *Gaillardia suavis* (Gray and Engelm) Britton and Rusby furnished no sesquiterpene lactones.

In the course of our study of *Helenium* and related species, we had occasion to examine *Gaillardia spathulata* Gray (*Compositae, Helenieae, Heleniinae*) a member of section *Eugaillardia* which, according to Biddulph,[3] is limited to Carbon and Emery Counties, Utah. This has led to the isolation of a new pseudoguaianolide which we have called spathulin. The discussion of its structure and its occurrence in several other *Gaillardia* species is the subject of this paper.

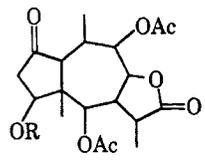
Spathulin ($C_{19}H_{26}O_8$, mp 261–262°, $[\alpha]^{25}D$ +17°), was obtained consistently in 0.15–0.2% yield from the dried, above-ground parts of *G. spathulata* Gray. The ultraviolet (strong end absorption) and infrared spectrum (bands at 1775 and 1650 cm^{-1}) suggested the presence of the α,β-unsaturated lactone chromophore (partial structure A) found in other sesquiterpene lactone constituents of *Gaillardia* species.[2,4-6] The empirical formula and infrared bands at 1740 and 1725 cm^{-1} indicated the possibility that two acetoxy functions might be present. Lastly, strong hydroxyl absorption in the infrared and relatively high polarity could be accounted for by assuming the presence of two hydroxyl groups.



1a, R = H
b, R = Ac



2a, R = H
b, R = Ac
c, R = Ms



3a, R = H
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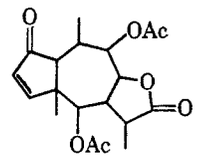
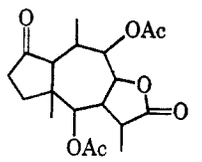


4a, b



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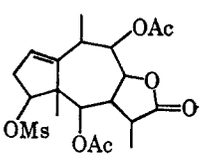

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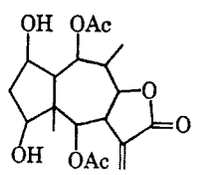

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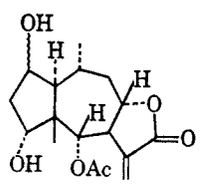

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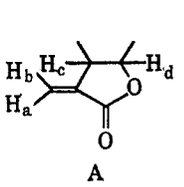
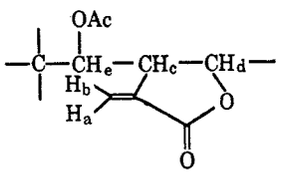
These assignments were partially verified by hydrogenation which revealed the presence of one double bond and resulted in the formation of dihydrospathulin (**2a**), by ozonolysis (liberation of formaldehyde) which indicated the presence of one exocyclic double bond, and by the formation of diacetyl derivatives **1b** and **2b** on treatment of **1a** and **2a** with acetic anhydride–pyridine.

The nmr spectra of these compounds confirmed the assignments. Diacetylspathulin (**1b**) exhibited two narrowly split signals at 6.40 and 5.89 ppm characteristic of an exocyclic methylene group conjugated with a lactone function. These were absent from the nmr spectrum of **2b** which, however, had three methyl groups (evidenced by two methyl doublets and one methyl singlet) as contrasted with **1b** which had only two (one doublet and one singlet). The spectrum of **1b** also contained a doublet at 5.56 ppm (chemical shift characteristic of hydrogen on carbon carrying one of the four acetoxyls clearly visible as four three-proton singlets) which was obviously spin coupled to only one adjacent proton.

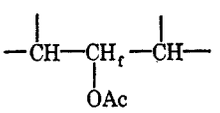
That the remaining three acetoxyls also esterified secondary alcohol functions was indicated by a complex, four-proton signal centered at 4.9 ppm which also embodied H_d of partial structure A. This could be shown by spin-decoupling experiments. Irradiation



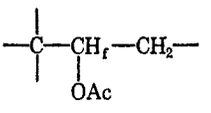
A



B



C



D

at 3.20 ppm (broad multiplet owing to H_c) resulted in the collapse of the vinylic doublets (H_a and H_b) to singlets and caused a change in the appearance of the complex, four-proton multiplet. Simultaneously, the doublet at 5.56 ppm collapsed to a sharp singlet. This permitted expansion of A to B where CH_e adjoins a fully substituted carbon atom unless the coupling constant between H_e and a second vicinal hydrogen atom is 0. The H_c signal had the appearance of a broadened

(1) Supported in part by grants from the U. S. Public Health Service (GM-05814) and the National Science Foundation (GP-1962).

(2) Previous paper: W. Herz, S. Rajappa, S. K. Roy, J. J. Schmid, and R. N. Mirrington, *Tetrahedron*, **22**, 1907 (1966).

(3) S. F. Biddulph, *Res. Studies Wash. State Coll.*, **12**, 195 (1944).

(4) W. Herz, K. Ueda, and S. Inayama, *Tetrahedron*, **19**, 483 (1963).

(5) W. Herz and S. Inayama, *ibid.*, **20**, 347 (1964).

(6) W. Herz, S. Rajappa, M. V. Lakshmikantham, and J. J. Schmid, *ibid.*, **22**, 693 (1966).

TABLE I
NMR SPECTRA OF SPATHULIN DERIVATIVES[a]

Compd	H_2	H_4	H_6	H_8	H_9	H_{13}	C_5-Me	C_{10}-Me	Misc
1a[b]	4.5[c]	4.5[c]	6.05 d (2.5)	4.5[c]	5.15 d, br (4)	6.20 d (3) 5.53 d (3)	0.67	1.07 d (5)	3.3,[d] 2.12,[e] 1.99[e]
1b	4.9[f]	4.9[f]	5.56 d (3)	4.9[f]	4.9[f]	6.40 d (3) 5.89 d (3)	0.94	0.99 d (7)	3.2,[d] 2.12,[e] 2.12,[e] 2.05[e] 2.05[e]
2b	4.9[f]	4.9[f]	5.42 br[g]	4.9[f]	4.9[f]	1.17 d (7)	0.82	0.94 d (7)	2.17,[e] 2.12,[e] 2.12,[e] 2.05[e]
3		4.12 dd	5.79 br[g]	4.75 c		1.31 d (7)	0.80	1.22 d (7)	2.12,[e] 2.12[e]
4a		7.37 d (5.5)	5.33 br[g]	4.55 t (10.5)	5.20 t (10)	1.33 d (7)	1.22	1.11 d (7)	6.26 d (5.5),[h] 2.17,[e] 2.10[e]
4d		7.27 d (5.5)	5.21 br[g]	4.25 t (10.5)	5.30 dd (12, 11)	1.36 d (7)	1.26	1.17 d (7)	6.25 d (5.5),[h] 2.26,[e] 2.11[e]
6	5.70 m	4.8[c]	5.60 br	4.8[c]	4.8[c]	1.18 d (7)	0.94	1.16 d (7)	3.08,[i] 2.16,[e] 2.16[e]

[a] Spectra were determined in deuteriochloroform solution on a Varian A-60 spectrometer using tetramethylsilane as internal standard, unless otherwise specified. Chemical shifts are in parts per million (ppm), signals being denoted in the usual way: d, doublet; t, triplet; c, complex band whose center is given; br, slightly broadened singlet. Unmarked signals are singlets. Figures in parentheses are line separations in cycles per second (cps). Signals in first six columns correspond to one proton, in sixth column to three protons except for **1a** and **1b**, in seventh and eighth column to three protons. [b] Run in dimethyl sulfoxide. [c] Approximate center of complex set of signals resulting from superposition of three protons. [d] H_7. [e] Acetate. [f] Approximate center of complex set of signals resulting from superposition of four protons. [g] $W_{1/2} = 3$. [h] H_3. [i] Mesylate.

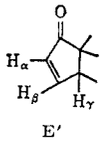
doublet, the large splitting being about 9 cps. Since $J_{H_cH_e}$, $J_{H_bH_c}$, and $J_{H_aH_c}$ are small, the larger coupling must be $J_{H_cH_d}$.

In the nmr spectrum of spathulin, the H_d resonance was superimposed on the signals generated by the protons α to the two hydroxyl groups. H_e was a sharp doublet at 6.05 ppm and the resonance of the proton α to the second acetoxyl function (H_f) had now emerged as a somewhat broadened doublet at 5.15 ppm. Its environment could therefore be represented by C or D.

The nature of the functional groups having been established, the empirical formula required that spathulin be bicyclic, exclusive of the γ-lactone ring. The relationship of the two hydroxyl groups was now dictated by the following series of transformations. Spathulin or its dihydroderivative (**2a**) did not react with periodic acid or lead tetraacetate. Selective oxidation of **2a** with Sarett's reagent furnished in modest yield dehydrodihydrospathulin **3a** which was not an α-ketol (negative periodic acid test). Comparison of the infrared spectrum of this substance with that of **2a** indicated that it was a cyclopentanone derivative (double intensity carbonyl band at 175 cm^{-1} owing to cyclopentanone and unchelated acetate, single intensity band at 1725 cm^{-1} owing to chelated acetate). Attention should also be drawn to the nmr spectrum (Table I) in which the lone HCOH resonance was now clearly visible as a doublet of doublets.

The hydroxyl group was eliminated by conversion of **3a** to the mesylate (**3b**) and subsequent treatment with base. This resulted in formation of two apparently epimeric cyclopentenones (**4a** and **4b**, new infrared band at 1710 cm^{-1}; λ_{max} 217 and 222 mμ, respectively), whose partial structure (E) was apparent from the nmr spectra. In particular, the doublet nature of the two vinyl protons near 7.3 (H_β) and 6.25 ppm (H_α) showed that the α and β positions were unsubstituted and that the γ carbon was quaternary.[7]

(7) The literature[4,8] shows that in cyclopentenones of type E′ H_β is inevitably coupled vicinally to H_γ as well as to H_β and that H_α is generally coupled allylically to H_γ. Hence partial structure E′ is excluded as a component of **4a** and **4b**.

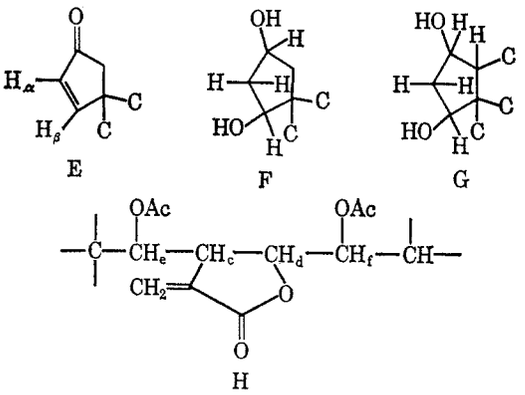


E′

(8) *Inter alia*, W. Herz, H. Watanabe, M. Miyazaki, and Y. Kishida, *J. Am. Chem. Soc.*, **84**, 3517 (1962); W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman, and N. Viswanathan, *ibid.*, **84**, 3857 (1962); W. Herz, A. Romo de Vivar, J. Romo, and H. Viswanathan, *ibid.*, **85**, 19 (1963); W. G. Dauben, K. Koch, S. J. Smith, and O. L. Chapman, *ibid.*, **85** 2617 (1963); C. H. DePuy, M. Isaks, K. L. Eilers, and G. F. Morris, *J. Org. Chem.*, **29**, 3503 (1964); Y. Inubushi, Y. Sasaki, Y. Tsuda, and J. Nakano, *Tetrahedron Letters*, 1519 (1965).

Catalytic hydrogenation of **4a** furnished a cyclopentanone (**5**, no carbonyl absorption below 1742 cm^{-1}).[9]

The above facts demonstrated that spathulin was a 1,3-cyclopentanediol of partial structure F. Chemical confirmation for this was provided by the formation of a sulfite which also indicated that the hydroxyls were *cis* related.



E F G

H

The environment of the second hydroxyl group was demonstrated as follows. Conversion of **2a** to the dimesylate (**2c**) was followed by treatment with lutidine. Only one mesyl group was eliminated and the nmr spectrum of the product (**6**) exhibited only one vinyl proton resonance. This permitted expansion of F to G which, since spathulin had one tertiary and one secondary methyl group, could be combined with B and C (or D) in only two ways, **1a** and **7**.

Formula **7** for spathulin was not only biogenetically implausible, but contraindicated by the behavior of **2c** under the influence of base. Vinylogous elimination of the acetate function and formation of a diene might have been expected to occur, had the second acetoxy group been at C-10 instead of at C-9. More convincing, perhaps, as an argument against **7** and in favor of **1a**, was the pronounced paramagnetic shift (Table I) of the C-10 methyl signal which accompanied oxidation of **2a** to **3** and **4a** or **4b**.

(9) The observed shifts in the infrared bands of one of the acetoxyl functions can be rationalized in terms of formula **1a** by assuming that the group attached to C-6 is hydrogen bonded, hence *cis* to the C-4 hydroxyl and appears at a lower than normal frequency. When the C-4 hydroxyl is blocked, as in **1b** or **2b**, or removed, as in **4** and **5**, the infrared frequency is normal and superimposed on the band of the acetoxyl group attached to C-9.

Clear evidence in favor of **1a** was finally secured by a double-resonance experiment involving **4b**. Irradiation at 4.25 ppm (H_d triplet) effected collapse of the 5.30 doublet of doublets (H_f) to a doublet. Hence H_d of B is coupled to H_f of C as in H and the structure of spathulin must unequivocally be **1a**.[10]

Spathulin appeared to be fairly widely distributed in *Gaillardia* species. Extraction of *G. aristata* Pursh. and *G. grandflora*, the latter a garden hybrid of *G. spathulata* and *G. aristata*, furnished spathulin and variable amounts of an unidentified new sesquiterpene lactone which we have called aristalin. Spathulin was the only crystalline substance isolated from a collection of the Rio Grande form of *Gaillardia pulchella* Foug., an observation which further demonstrates the great variability of this species.[11] *G. mexicana* Gray yielded spathulin and an unidentified lactone. Extraction of *G. parryi* Greene furnished flexuosin A (**8**)[12,13] previously isolated from *Helenium flexuosum* Raf.,[13] but *G. suavis* (Gray and Engelm.) Britton and Rusby appeared to be devoid of lactonic constituents.

Experimental Section[16]

Extraction of *Gaillardia spathulata* Gray.—Dried, aboveground plant, collected by Dr. Stewart C. Harvey in early June, 1960, along State Road 20 between Green River and Hanksville, Emery County, Utah, approximately 10 miles south of the junction with U. S. 6–50 (3150 g), was extracted with chloroform for 2 days. The chloroform was removed at reduced pressure, the residue was taken up in 600 ml of warm ethanol, diluted with 600 ml of water containing 25 g of lead acetate and a little acetic acid, allowed to stand for 2 days, and filtered. The filtrate was concentrated at reduced pressure and extracted thoroughly with chloroform. The washed and dried chloroform extract was evaporated *in vacuo*.

The residual crude gum (62 g) was taken up in chloroform–benzene (3:1) and chromatographed over 600 g of alumina (Alcoa F-20). Elution with chloroform–benzene and chloroform furnished only gummy material. Elution with chloroform–methanol (49:1, ten 500-ml fractions) gave solid which was recrystallized from acetone–petroleum ether to yield 5.5 g (0.17%) of spathulin, mp 259–261°. The analytical sample had mp 261–262°; $[\alpha]^{25}D$ +17° (*c* 1.0, 95% ethanol); infrared bands at 3650 and 3400 (nonbonded and bonded OH), 1775 (γ-lactone), 1740, 1725, and 1250 (two acetates), and 1650 cm^{-1} (double bond); λ_{max} 209 mμ (ε 16,100).

Anal. Calcd for $C_{19}H_{26}O_8$: C, 59.67; H, 6.85; O, 33.47. Found: C, 59.57; H, 6.85; O, 33.47.

Extraction of 1800 g of *G. spathulata* collected by Dr. W. P. Stoutamire on July 6, 1962, along the same road 6 miles south of the junction with U. S. 6-50 (WPS H4001) yielded 30 g of crude gum from which 3.6 g of recrystallized spathulin (0.2%) was isolated by chromatography.

Diacetylspathulin (1b).—A solution of 0.2 g of **1a** in 1 ml of pyridine was mixed with 2 ml of acetic anhydride, warmed slightly, left overnight at room temperature, and then poured on ice. The solid which separated was recrystallized from acetone–petroleum ether to yield 0.12 g: mp 252–254°, $[\alpha]^{25}D$ −26.8° (*c* 2.5), infrared bands at 1775 (γ-lactone), 1740 and 1250 (very strong, acetates), and 1660 cm^{-1} (double bond).

Anal. Calcd for $C_{23}H_{30}O_{10}$: C, 59.22; H, 6.48; O, 34.30. Found: C, 58.84; H, 6.65; O, 34.51.

Dihydrospathulin (2a).—A solution of 0.2 g of **1a** in 50 ml of ethanol was reduced with 40 mg of platinum oxide at a hydrogen pressure of 30 psi. After 4 hr, the solution was filtered and evaporated *in vacuo*. The residue was recrystallized from acetone–petroleum ether to yield 0.15 g of **2a**, mp 223–225°, completely clearing at 235°. The melting point of **2a** was variable in different runs, but in every instance the purity and identity of the reduction product were checked by thin layer chromatography, infrared spectrum, and preparation of the diacetate (*vide infra*). The analytical sample had $[\alpha]D$ ~0° (*c* 1.0, ethanol); infrared bands at 3600 and 3400 (bonded and nonbonded OH), 1780 (γ-lactone), and 1745, 1720, 1260, and 1250 cm^{-1} (two acetates).

Anal. Calcd for $C_{19}H_{28}O_8$: C, 59.36; H, 7.34; O, 33.30. Found: C, 59.01; H, 7.54; O, 33.08.

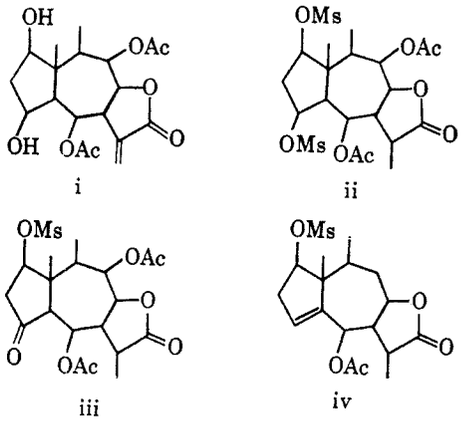
Diacetyldihydrospathulin (2b).—Acetylation of 0.2 g of **2a** in the usual manner furnished, after recrystallization from acetone–petroleum ether, 0.11 g of **2a**: mp 290–291°, infrared bands at 1780 (γ-lactone) and 1740 and 1240 cm^{-1} (very strong, acetates).

Anal. Calcd for $C_{23}H_{32}O_{10}$: C, 58.96; H, 6.89; O, 34.15. Found: C, 58.87; H, 7.07; O, 34.34.

Dehydrodihydrospathulin (3a).—To an ice-cold solution of pyridine–chromic oxide complex prepared from 10 ml of dry pyridine and 0.8 g of chromic acid was added slowly with stirring a solution of 0.4 g of **2a** in 5 ml of pyridine. Stirring was continued for 3 hr, and the mixture was left at room temperature for an additional 17 hr, poured on ice and extracted with ether. The washed and dried ether extract was evaporated and the residue was triturated with dry ether. This furnished 0.13 g of crude **3a** which was recrystallized from benzene–petroleum ether. The product had mp 186–188°; a positive Zimmermann test; infrared bands at 3475 (OH); 1785 (γ-lactone), 1745 (double-intensity cyclopentanone and C-9-acetate), and 1720 cm^{-1} (C-6-acetate); ORD curve (*c* 0.064, methanol) $[\alpha]_{318}$ +984°, $[\alpha]_{275}$ −734°.

Anal. Calcd for $C_{19}H_{26}O_6$: C, 59.67; H, 6.85; O, 33.47. Found: C, 59.96; H, 6.70; O, 33.02.

Anhydrodehydrodihydropathulin (4a and 4b). A.—A solution of 0.2 g of **3a** in 1.5 ml of pyridine was treated with 0.5 g of methanesulfonyl chloride in the cold, kept at 5° for 2 hr, and poured on ice. The crude mesylate (**3b**) was washed with water and dried; the infrared spectrum indicated the absence of hydroxyl. It was taken up in 10 ml of ethanol, refluxed with 0.4 g of freshly



(10) A referee has suggested that the biogenetically quite implausible formula (i) should also be considered since $J_{H_5H_6}$ might be fortuitously 0 in all compounds of the spathulin series. In view of the profound chemical alterations which are effected during the various transformations and must undoubtedly be accompanied by conformational changes that would produce changes in $J_{H_5H_6}$, we considered such a possibility extremely unlikely. Moreover, one would expect elimination of the acetate function on base treatment of ii and iii, the only permissible alternatives to **2c** and **3b**. This was not observed. Thirdly H_6 of iv, the only permissible alternative to **6**, would be expected to experience considerable deshielding relative to the position of the H_6 signal in the other compounds. This was not the case as seen from Table I. Lastly the nmr spectrum of **6** displayed only two acetate and three methyl resonances in the region 1–2.2 ppm where the signal corresponding to H_{10} is ordinarily found as well. On the other hand a complex series of signals integrating for five protons was found at 2.3–3.0 ppm. This must therefore include the resonance of H_{10} which is obviously deshielded as demanded by **6** but not by iv.

(11) Pulchellin is the main constituent[2] of the coastal race of *G. pulchella*. Collections of *G. pulchella* from New Mexico and Arizona yielded pulchellin B^3, C^3, and D^3 as well as other lactones: S. K. Roy, unpublished results.

(12) W. Herz, Y. Kishida, and M. V. Lakshmikantham, *Tetrahedron*, **20**, 979 (1964).

(13) W. Herz and H. B. Kagan, *J. Org. Chem.*, **32**, 216 (1967). Absolute configurations at C-6, C-7, and C-8 are tentative and are assigned on the basis of the conversion of flexuosin A to what appeared to be 2,3-dihydrobigelovin[14] whose absolute configuration is now known.[15]

(14) B. A. Parker and T. A. Geissman, *ibid.*, **27**, 4127 (1962).

(15) W. Herz and M. V. Lakshmikantham, *Tetrahedron*, **21**, 1711 (1965).

(16) Melting points are uncorrected. Analyses were by Dr. F. Pascher, Bonn, Germany. Ultraviolet spectra were run in 95% ethanol, infrared spectra were run as KBr pellets, rotations were determined in chloroform, and nmr spectra were determined in deuteriochloroform unless otherwise specified. Petroleum ether boiled at 30–60°.

fused sodium acetate for 2 hr, and evaporated at reduced pressure. The residue was diluted with water, extracted with ether, and washed, and the dried extracts were evaporated. The residue solidified on trituration with a small amount of ether, but recrystallization from ethyl acetate–petroleum ether afforded only 8 mg of **4a**. The mother liquors were therefore combined, evaporated, taken up in chloroform, and chromatographed over acid-washed alumina. Elution with chloroform furnished crude **4a** which was recrystallized from ethyl acetate–petroleum ether. The product (80 mg) had mp 174–177°; λ_{max} 222 mμ (ε 8340); infrared bands at 1780 (γ-lactone), 1742 (double intensity, acetates), and 1710 cm^{-1} (cyclopentenone).

Anal. Calcd for $C_{19}H_{24}O_7$: C, 62.62; H, 6.64; O, 30.73. Found: C, 62.65; H, 6.70; O, 30.80.

B.—In a second, larger preparation, crude **3a** (2 g), prepared from 4.5 g of spathulin without purification of **2a**, was converted to the mesylate and the latter refluxed with 4 g of freshly fused sodium acetate and 60 ml of ethanol for 3.5 hr, kept at room temperature overnight, and worked up as described in the preceding paragraph. Chromatography of the crude gum (1.05 g) over acid-washed alumina gave, on elution with chloroform, a solid (0.88 g), which was recrystallized from ethyl acetate, had mp 170–177°, and showed two spots on tlc. Rechromatography over 50 g of silica gel (eluents chloroform, chloroform–ethyl acetate, and ethyl acetate) gave in the chloroform eluate 0.3 g of **4b**: mp 220–222° after recrystallization from methanol; $[\alpha]^{25}D$ +50° (*c* 1.1); λ_{max} 217 mμ (ε 5200); infrared bands at 1780 (γ-lactone), 1742 (two acetates), and 1710 cm^{-1} (cyclopentenone).

Elution with ethyl acetate furnished 0.15 g of **4a** which after recrystallization from ethyl acetate–petroleum ether now melted at 182–184° and had $[\alpha]D$ 0° (*c* 1.0), but was identical with the material described in section A (nmr, infrared spectrum, tlc).

Dihydroanhydrodehydrodihydrospathulin (5).—Catalytic reduction of 0.1 g of **4a** in 15 ml of ethanol with 20 mg of 5% palladium–charcoal at atmospheric pressure furnished, after recrystallization from ethyl acetate–petroleum ether, 50 mg of **5** mp, 173–174°; mixture melting point with **4a** depressed to about 160–165°; infrared bands at 1785 (γ-lactone) and 1742 cm^{-1} (strong, two acetates and cyclopentanone); ORD curve (3.4 mg) in 5 ml of methanol $[\alpha]_{322.5}$ −1035°, $[\alpha]_{300}$ +40 (inflex), $[\alpha]_{284}$ +1200.

Anal. Calcd for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15; O, 30.57. Found: C, 62.29; H, 7.26; O, 30.58.

$\Delta^{1,2}$-Anhydrodihydrospathulin Mesylate (6).—A solution of 0.2 g of **2a** in 1.5 ml of pyridine was mixed with 0.5 ml of methanesulfonyl chloride in the cold and kept in the refrigerator overnight, and then poured on ice. The precipitated dimesylate was washed well with water and dried, and without further purification was refluxed with 3 ml of lutidine for 4 hr, cooled, poured on ice, and extracted with ether. The washed and dried ether extract was evaporated and the residue was chromatographed over acid-washed alumina. Elution with benzene removed coloring matter. Subsequent elution with benzene–ether (1:1) and digestion of the residue with dry ether furnished 20 mg of a solid which was recrystallized from benzene–petroleum ether and had mp 170–172° dec; infrared bands at 1780 (γ-lactone) and 1745 cm^{-1} (two acetates).

Anal. Calcd for $C_{20}H_{28}O_9S$: C, 54.05; H, 6.35. Found: C, 54.28; H, 6.80.

Dihydrospathulin Sulfite (9).—A solution of 50 mg of **3** in 1 ml of dry pyridine was mixed with 4 drops of thionyl chloride at ice temperature, left at room temperature for 4 hr, and then poured on ice. The solid was washed with water, dried, and recrystallized from benzene–petroleum ether. The product (15 mg) had mp 175–178° dec; infrared bands at 1785 (γ-lactone), 1745 and 1240 (double intensity, two acetates), and 1030 cm^{-1} (sulfite).

Anal. Calcd for $C_{19}H_{24}O_9S$: C, 53.02; H, 6.09; S, 7.43. Found: C, 53.21; H, 6.75; S, 7.81.

Extraction of *Gaillardia aristata* Pursh.—Above-ground, dried, whole plant (1175 g), collected near Gold Hill, Boulder County, Colo., in July and August 1957, was extracted with chloroform and worked up in the usual fashion to yield 21 g of crude gum. Half of this material was stirred with low-boiling petroleum ether, dissolved in 30 ml of chloroform, chromatographed over 175 g of alumina (Alcoa F-20), and eluted successively with chloroform (seven 150-ml fractions), chloroform–methanol (49:1, 20 fractions), and chloroform–methanol (11:1, eight fractions). Fractions 9–21 gave semisolids. Treatment of fractions 9 and 10 with ethyl acetate–petroleum ether–ether furnished 0.16 g of crystalline material, mp 255–256°, identical with spathulin by mixture melting point and comparison of infrared spectra. Fractions 11–14 could not be crystallized satisfactorily. Fractions 15–31 on solution in ethyl acetate and treatment with ether furnished a new polar lactone, aristalin, which was recrystallized from acetone–ether–petroleum ether and acetone–benzene–petroleum ether. Aristalin, obtained in 0.15-g yield, had mp 204–206°; infrared bands at 3700 (strong complex OH), 1770 (γ-lactone), 1710 (conjugated ester), and 1660 cm^{-1} (conjugated double bond); nmr signals (in deuterioacetone–deuteriodimethyl sulfoxide) at 6.20 d [3, *H*COC=O)] superimposed on multiplet centered at 6.15 (vinyl H of side chain) complex signals from 5.3 to 4.5, intensity four protons, 2.08 dm and 1.88 (vinyl methyls of side chain), 1.05 d (8), and 0.92 d (7, C_{10} and C_{11} methyls), and 0.70 ppm (C_5 methyl).

Anal. Calcd for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Calcd for $C_{20}H_{30}O_7$: C, 62.81; H, 7.91. Found: C, 63.18, 63.30; H, 7.69, 7.56.

Extraction of *Gaillardia grandiflora*.—Above-ground, dried, whole plant, a garden hybrid of *G. aristata* Pursh. and *G. pulchella* Foug. (390 g), grown in 1959 by Dr. W. P. Stoutamire from seed collected at the Boyce Thompson Arboretum, Superior, Ariz. (WPS No. 2543), was extracted and worked up in the usual manner. The crude gum (20 g) was taken up in 25 ml of chloroform, chromatographed over 200 g of alumina (Alcoa F-20), and eluted with chloroform (seven 150-ml fractions), chloroform methanol (49:1, six fractions), chloroform–methanol (11:1, eight fractions), and chloroform–methanol (5:1, two 250-ml fractions). Fractions 11–23 gave semisolid residues. The material from fractions 13–15 on recrystallization from acetone and ether gave 0.09 g of spathulin, mp 255–256°, identical with authentic spathulin by mixed melting point and comparison of infrared spectra. Treatment of fractions 16–21 with ether gave 0.35 g of solid, which after recrystallization from acetone–benzene–petroleum–ether melted at 204–206° and was identical with aristalin by mixture melting point and comparison of infrared spectra.

Extraction of *Gaillardia Pulchella* Foug. (Rio Grande Form).—Dried, above-ground material (3 lb) grown in 1962 by Dr. W. P. Stoutamire in the Cranbrook Institute of Science Experimental Garden from seed of races 2502–2505 collected along U. S. 90 between Devil's River and Langtry, Val Verde, Texas, was ground, extracted with chloroform, and worked up in the usual way. The gummy residue (9 g) was chromatographed over 250 g of Alcoa F-20 alumina. Benzene–chloroform (1:3, seven 120-ml fractions) eluted 0.6 g of gum which appeared to be homogeneous (thin layer chromatography showed the absence of pulchellin and pulchellin C) but could not be crystallized. Chloroform (eight 120-ml fractions) eluted a trace of material. Chloroform–methanol (9:1, 200 ml) eluted 2.6 g of gum which again contained no pulchellin or pulchellin C (tlc). This was rechromatographed over 50 g of acid-washed alumina using chloroform and chloroform–methanol (20:1). The fractions exhibiting identical R_f values were pooled. From the middle fractions was isolated after recrystallization, approximately 0.2 g of spathulin, mp 260°, identified by R_f value, mixed melting point with an authentic sample and infrared spectrum.

Extraction of *Gaillardia suavis* (Gray and Engelm.) Britton and Rusby.—Above-ground, dried plant material (520 g), grown in 1959, from seed collected by Dr. W. P Stoutamire at localities in Coahuila (Mexico), Texas, and Oklahoma (WPS No. 2150–2152, 2440, 2450, 2494, 2496, 2510, 2683, and 2686), was extracted with chloroform and worked up in the usual way. The yield of crude gum was only 1.1 g, presumably because *G. suavis* completely lacks the epidermal resiniferous glands characteristic of other *Gaillardia* and *Helenium* species (private communication from Dr. Stoutamire). Chromatography of this material failed to yield crystalline fractions. Repetition of the extraction with 1150 g of dry plant material collected in 1960 by Dr. Stoutamire in East Texas yielded only 2.5 g of gum which again could not be crystallized.

Extraction of *Gaillardia mexicana* Gray.—Above-ground, dried plant (350 g), grown in 1959, from seed collected by Dr. W. P. Stoutamire at Lerios, Coahuila, Mexico (WPS No. 2466 and 2810), was extracted with chloroform and worked up in the usual manner. The residual gum (3.5 g) was dissolved in 45 ml of benzene–chloroform (1:1) and chromatographed over 45 g of alumina (Alcoa F-20) with benzene–chloroform (1:1, two 50- and three 75-ml fractions) and chloroform (500 ml). Fractions

1 and 2 solidified (0.3 g) and were recrystallized from acetone–benzene–petroleum ether to give a new sesquiterpene lactone which had mp 203–206°, infrared bands ($CHCl_3$) at 3700 and 3500 (broad, OH), 1770 (γ-lactone), 1725 (broad, double-intensity), shoulders at 1660 and 1650 cm^{-1} (double bands); intense end absorption at 205–210 mμ. Fractions 3 and 4 yielded additional small quantities of this substance.

Anal. Calcd for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 62.88; H, 7.32.

Fraction 6 on solution in acetone and addition of petroleum ether yielded 0.1 g of crystalline material which after recrystallization from acetone–ether–petroleum ether melted at 255–256° and was identified as spathulin.

Extraction of *Gaillardia parryi* Greene.—Dried, above-ground material (3 lb), collected in July 1962, by Dr. W. P. Stoutamire (WPS No. 3991) along Utah State Road 64 between St. George, Utah, and Wolf Hole, Ariz., a few miles south of the Arizona line was extracted with chloroform and worked up as usual. The residual crude gum (25 g) was dissolved in benzene–chloroform (1:3) and chromatographed over 350 g of alumina (Alcoa F-20, washed with ethyl acetate and dried at 140° for 1 hr), using six 200-ml fractions of benzene–chloroform (8.3 g of gum), seven 200-ml fractions of chloroform (2.9 g of solid which was homogeneous by thin layer chromatography), and chloroform–methanol (9:1, 1.1 g of solid identical with the previous material). Recrystallization of the solid fractions from acetone–isopropyl ether furnished 3.1 g of flexuosin A, mp 225°, identical with authentic material by R_f, mixture melting point, and infrared spectrum. Repeated rechromatography of the first eluate gave largely gummy material and in the more polar fractions additional small amounts of flexuosin A.

Registry No.—**1a**, 7706-45-8; **1b**, 7699-92-5; **2a**, 7699-93-6; **2b**, 7699-94-7; **3a**, 7699-95-8; **4a**, 7699-96-9; **5**, 7699-97-0; **6**, 7699-98-1; flexuosin A, 1381-28-8; **9**, 10035-79-7.

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A Total Synthesis of Optically Active Lupinine without Benefit of Resolution

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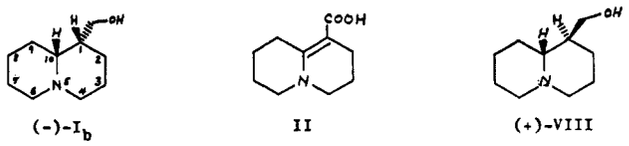
Synthesis of the optically active natural product lupinine has been achieved directly, without a separate resolution step. Sodium borohydride reductions of (−)- and (+)-1-menthoxycarbonyl-1(10)-dehydroquinolizidines gave (−)- and (+)-1-menthoxycarbonylquinolizidines, respectively, as the predominant isomers. Subsequent treatment with lithium aluminum hydride yielded (+)- and (−)-lupinines, respectively, in near 10% optical yields. Deuterium-labeling experiments showed C-10 in the unsaturated esters to be the site of hydride delivery. The observed asymmetric selection and the configurational relationships involved are accounted for with topological transition state models.

The absolute configuration of (−)-lupinine was established as (1*R*,10*R*)-1-hydroxymethylquinolizidine (Ib),[1] and, while racemic lupinine has been synthesized in a number of laboratories,[2] we have chosen this system, with its two asymmetric carbons, as the goal of a total synthesis which would include, in the chemical reactions used to attain the desired bonding sequence, features to impart asymmetric selection, so that the synthetic material might be produced in a predominant enantiomeric form without a separate resolution step. The concept required development of a synthesis scheme which would include an intermediate containing an atom, not to become part of the lupinine bonding sequence, but with a fixed configurational arrangement which could be used to influence the configuration of one of the asymmetric centers (C-1 or C-10) in lupinine as the latter was formed. The new asymmetric atom, thus formed in a predominant configuration, would then in turn influence formation of the second asymmetric atom in lupinine. Finally, the originally present atom of fixed configuration would subsequently be removed en route to lupinine. We concluded that an optically active ester of 1-carboxy-1(10)-dehydroquinolizidine (II) would fulfill these requirements, and we were able to obtain optically active synthetic lupinine in this manner.



(-)-I_b II (+)-VIII

The enantiomeric 1-menthoxycarbonyl-1(10)-dehydroquinolizidines (IIIa and IIIb) were individually prepared (Scheme I) by allowing 1(10)-dehydroquinolizidine (IV) to react with each of the enantiomeric menthyl chloroformates (Va and Vb). The details of the preparation of quinolizidine (VI), from which IV was prepared by mercuric acetate dehydrogenation[3] of the former, are given in the Experimental Section. Also given are the details of the preparations of each enantiomeric menthyl chloroformate (Va and Vb) which were obtained *via* reaction of enantiomeric menthol with phosgene in the presence of quinoline.

Treatment of each of enantiomeric unsaturated esters (−)-IIIa and (+)-IIIb with sodium borohydride in methanol (Scheme II) produced the saturated esters, (−)-VIIa and (+)-VIIb, respectively. The predominant isomer obtained from reduction of (−)-IIIa was (−)-VIIa, while (+)-VIIb was the predominant product obtained from (+)-IIIb. These assignments were clearly established by means of conversion of

(1) For an account of the experimental work upon which this was based, see N. J. Leonard in "The Alkaloids," R. H. F. Manske, Ed., Academic Press Inc., New York, N. Y., 1960, pp 264, 265.

(2) G. R. Clemo, W. McG. Morgan, and R. Raper, *J. Chem. Soc.*, 965 (1937); K. Winterfeld and H. von Cosel, *Arch. Pharm.*, **278**, 70 (1940); V. Boekelheide and J. P. Lodge, Jr., *J. Am. Chem. Soc.*, **73**, 3681 (1951); V. Boekelheide, W. J. Linn, P. O'Grady, and M. Lamborg, *ibid.*, **75**, 3243 (1953); J. Ratusky and F. Sorm, *Chem. Listy*, **47**, 1491 (1953); *Collection Czech. Chem. Commun.*, **19**, 340 (1954); H. R. Lewis and C. W. Shoppee, *J. Chem. Soc.*, 313 (1956).

(3) N. J. Leonard, A. S. Hay, R. W. Fulmer, and V. Gash, *J. Am. Chem. Soc.*, **77**, 439 (1955).